Research Article

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Homozygosity and segregation ratio in F_4 generation of tomato for fruit morphology

Abstract

Diversity in tomato shape is one of the most prominent traits which distinguish one variety of tomato from other. Our research aims to find the segregation ratio for fruit morphology in F_4 lines and to determine the level of homozygosity within the lines. Plants with desirable traits were selected from F_3 generation in 2017 and were sown as F_4 generation in next season i.e 2018. Data were recorded for each plant in each line for fruit and blossom end shape and were analyzed through chi square test. Chi square test showed that more than 50% lines deviated from the expected ratio for fruit shape and showed significant difference between expected and observed ratios. Most of the obovoid-square fruit shaped F_3 parents did not segregate further and produced all the obovoid-square shaped fruit plants in F_4 generation. Obovoid fruit shaped F_3 parents segregated into different fruit shapes in different ratios and did not show any homozygosity in F_4 generation. Obovoid-pear fruit shaped F_3 parents segregated into the Obvoid and pear shapes. Obovoid-cylindrical fruit shaped F_3 parents did not produce any cylindrical fruit in F_4 generation and segregated into square and obovoid shaped fruit plants. However, for blossom end shape, nearly all the lines segregated in F_4 generation into flat blossom end and pointed blossom end shapes in the expected ratio i.e 3:1, respectively. Some lines did not segregate further for blossom end shape showing that those lines have attained homozygosity in F_4 generation for the trait.

Keywords: tomato, F_4, segregation, ratio, fruit, shape, blossom-end

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Introduction

Tomato fruit shape is an important feature which determines its marketing and processing value.[1] Fruit shape and related traits are clear and visible phenotypic markers which help determine the genotypic compositions of the breeding generations. In tomato breeding programme, segregation can be clearly observed in fruit shape more than other traits like: leaves, flower etc. The study of segregation in fruit shape in Fn generations determines whether the inbreeding lines have attained homozygosity or they are still in process of segregation.

Tomato has diversity in fruit shapes viz, rectangular, round, ovate, obovoid, and cylindrical etc.[2] Diversity in fruit shape in cultivated germplasm are attributed to a great extent to four genes.[3] These four major genes include: FAS which increases locule number and size,[4] LC which increases locule number and fruit size,[5] OVATE which gives obovoid fruit shape[6] and SUN which gives an elongated fruit shape[7] or the oxheart shape when associated to LC and FAS. However, elongated fruit shape is controlled by only one major locus.[8]

The study of tomato breeding lines at different stages helps in understanding the gene actions and genes responsible for tomato fruit morphology. The level of homozygosity for next breeding generations can also be determined by studying the lines for fruit morphology in breeding generations. There is dire need in tomato breeding programme to find at which stage tomato lines attain homozygosity for fruit shape and other parameters and to make selection on the basis of fruit shape according to preference of the local community. In our research programme, we aim to study the fruit and related characteristics and fix the desired fruit shape in later breeding generations.

Material and methods

In the year 2014, cross was made between two varieties: Roma (pear shaped fruit and semi pointed blossom end) and KHT5 (Obovoid-square shaped fruit and flat blossom end).The seed was extracted from the crossed-fruits and F_1 generation was developed in next year i.e 2015. The F_2 generation was advanced from F_1 in year 2016.The selection was made in F_2 generation and seeds were extracted from the selected plants to proceed to F_3 generation. In Year 2017, F_3 tomato lines were sown as nursery and 45 days plantlets were transplanted in the field and data were recorded on fruit traits. The plants with desired traits were selected from the F_3 lines on individual basis and data for fruit morphology were recorded from the selected plants and seeds were extracted to develop F_4 breeding lines. In next growing season i.e 2018, these selected F_4 lines were sown as nursery in separate pots and after 45 days those lines were transplanted in the field in separate rows. Row to row distance was maintained to be 100cm and plant to plant distance was kept to be 50 cm. On fruit maturity, morphological data on fruits were collected from each plant of each line. The fruits were harvested on ripening and seeds were extracted.

Data were collected from each plant of each line for the following traits:

A. Fruit shape

B. Blossom-end shape.

Each plant was carefully observed for the collection of data on the above parameters.

Statistical analysis

Data were analyzed using the chi square test according to formula given in the below lines. 9:3:3:1 ratio was set as expected ratio for obovoid-square, square, obovoid and pear shape, respectively. While 3:1 ratio was set for flat and pointed blossom end.

$X^2 = \sum (\text{Observed} - \text{expected})^2/\text{expected}$

Where X^2 is chi square

The chi square values were compared with the values in chi square table and the hypothesis was accepted or rejected on the basis of those values.

Results and discussion

Fruit shape

Chi square test shows that 13 of the 24 F_4 lines deviated from the expected ratio i.e 9:3:3:1 (P>0.01) for fruit shape, while 11 lines followed the expected pattern of ratio for segregation for fruit shape (Table.1).

Table 1 Homozygosity %, expected ratio and observed ratio of segregation and chi square values for F_4 lines of tomato for fruit shape

Entry Code	F3 parents	Homozygosity	Expected ratio	Observed ratio	Chi square	Sig/NS at 0.01
	Fruit shape	%	Sq-obv:Sq:Obv:Pear (9:3:3:1)	Sq-obv:Sq:Obv:Pear	X2	
RK1	Obv.Sq	100%	5.6:1.8:1.8:0.6	0 : 10 : 0 : 0	43.45	Sig
RK2	Obv.Sq	70%	5.6:1.8:1.8:0.6	7 : 0:3:0	3.51	NS
RK3	Obv	90%	5.6:1.8:1.8:0.6	1:0:9:0	33.47	Sig
RK4	Obv	60%	5.6:1.8:1.8:0.6	3:1:0:6	50.18	Sig
RK5	Obv.Sq	100%	5.6:1.8:1.8:0.6	10:0:0:0	7.77	NS
RK6	Obv.Sq	100%	5.6:1.8:1.8:0.6	10:0:0:0	7.77	NS
RK7	Obv.Sq	100%	5.6:1.8:1.8:0.6	10:0:0:0	7.77	NS
RK8	Obv.Sq	100%	5.6:1.8:1.8:0.6	10:0:0:0	7.77	NS
RK9	Obv.Sq	100%	5.6:1.8:1.8:0.6	10:0:0:0	7.77	NS
RK10	Obv.Sq	100%	5.6:1.8:1.8:0.6	10:0:0:0	7.77	NS
RK11	Obv-pear	70%	5.6:1.8:1.8:0.6	0:0:3:7	73.82	Sig
RK12	Obv.Sq	100%	5.6:1.8:1.8:0.6	10:0:0:0	7.77	NS
RK13	Obv.Sq	60%	5.6:1.8:1.8:0.6	3:1:6:0	11.36	Sig
RK14	Obv	60%	5.6:1.8:1.8:0.6	0:6:4:0	17.78	Sig
RK15	Obv	70%	5.6:1.8:1.8:0.6	0:3:7:0	20.99	Sig
RK16	Obv	80%	5.6:1.8:1.8:0.6	8:0:2:0	3.5	NS
RK17	Sq.cyl	50%	5.6:1.8:1.8:0.6	1:1:3:5	35.82	Sig
RK18	Obv	70%	5.6:1.8:1.8:0.6	1:2:7:0	18.5	Sig
RK19	Obv	40%	5.6:1.8:1.8:0.6	1:1:4:3	15.76	Sig
RK20	Obv	50%	5.6:1.8:1.8:0.6	4:1:5:0	6.73	NS
RK21	Obv	50%	5.6:1.8:1.8:0.6	1:5:4:0	12.08	NS
RK22	Obv.Cyl	80%	5.6:1.8:1.8:0.6	0:2:8:0	26.34	Sig
RK23	Obv.Sq	50%	5.6:1.8:1.8:0.6	3:0:2:5	34.04	Sig
RK24	Obv .Sq	50%	5.6:1.8:1.8:0.6	0:5:5:0	16.71	Sig

Obv-sq, obovoid-square; Obv, obovoid; Obv-Cyl, obovoid-cylindrical; sq-cyl, square- cylindrical; NS, non significant; Sig, sigificant at alpha level 0.01

Most of the F_3 lines having fruit shape Obovoid-square i.e RK1,RK5,RK6,RK7,RK8,RK9,RK10 and RK12 produced all the obovoid- square shaped fruits in F_4 generation and did not show segregation for other fruit shapes. The chi square test showed non-significant difference between expected and observed ratios of segregation (Table 1). The results show that the lines were completely homozygous for the trait.

F_4 progeny of some F_3 parental lines (obovoid-square) showed significant difference between observed and expected ratio. Among those lines RK2 segregated into 7:3 (Obovoid-square: square) as shown in Table 1. The ratio shows that the parental lines are heterozygous for fruit shape at F_3 stage and obovoid-square genes are dominant over square shaped genes. RK13 segregated into obovoid-Square, square and obovoid shapes in the ratio of 3:1:6 (Table 1). There is trend of recessiveness from obovoid shape to obovoid-square and from obovoid-square shape to square shape as clear from the above findings. The line RK23 segregated into obovoid-square, obovoid and pear shape in 3:2:5 ratios (Table 1), respectively. The line RK24 segregated into square and obovoid shapes in the ratio of 5:5 (Table 1). The parental line RK1 produced only square shape fruits in F_4 generation and did not segregate for other fruit shapes.

The unexpected fruit shapes in F_4 may be due to allelic variation in *sun* and *fs8·1* loci that can cause elongated and square fruit shapes, respectively.[9,10]

All the obovoid shaped F_3 parents segregated into different combinations in F_4 generation and did not show homozygosity in any line which depicts those obovoid shaped genes were dominant over obovoid-square, square and pear shaped genes in F_3. Some of the F_4 progeny of these F_3 lines showed significant difference between expected and observed ratio. The line RK3 segregated into square-obovoid and obovoid shapes in the ratio of 1:9. The line RK4 segregated into square-obovoid, square and pear shape in the ratio of 3:1:6, respectively (Table 1). The lines RK14 and 15 segregated into square and obovoid shapes in the ratio of 6:4 and 3:7, respectively. The line RK16 segregated into obovoid -square and obovoid shapes in the ratio of 8:2 and the chi square test shows that difference between observed and expected ratio was non-significant (Table 1). The lines RK20 and 21 whose F_3 parents were obovoid shaped segregated into square- obovoid, square and obovoid fruit shape in different ratios i.e 4:1:5 and 1:5:4, respectively (Table 1). Unexpected ratios in fruit shapes from different parents of the same shape may be attributed to the cause as discussed by Gustavo et al.,[11] who found that interactions between genes and uncharacterized modifiers also affect fruit shape: some lines have the duplication of the *SUN* gene and develop an ellipsoid instead of a long-shaped fruit. Moreover, differences in fruit shape of varieties carrying the *OVATE*, *FAS*, and *LC* mutations shows that suppressors and enhancers of these genes are present within the cultivated germplasm. For example, accessions that carry the *OVATE* mutation display a range of fruit shapes from long and obovoid to round whereas accessions carrying *LC* mutation produce long, oxheart, round, or flat fruit.[12]

The F3 parents having obovoid-pear fruit shape segregated into obovoid and pear fruit shape in the ratio of 3:7 and it showed significant difference between the observed and expected ratio. Butler,[13] found that O gene is responsible for both ovate and pear shape. Wua et al.,[14] also reported that ovate alleles can be found in obovoid and ellipsoid varieties; which confirms the above statement. The F3 parents RK17 (square-cylindrical) and RK22 (Obovoid-Cylindrical) showed significant difference between observed and expected values for fruit shape and segregated into square, Obovoid-square, obovoid and pear shapes in the ratio of 1:1:3:5 and 0:2:8:0, respectively (Table 1). The ovate locus contributes in the formation of pear and cylindrical shapes[15] therefore; cylindrical shaped parents can produce pear shaped progeny.

Blossom end shape

All the 24 lines showed non-significant difference between observed and expected values except the lines RK-16 and RK22 (Table 2).

Table 2 Homozygosity %, expected ratio and observed ratio of segregation and chi square values for F_4 lines of tomato for pointed and flat blossom end shape of tomato

Entry code	F3 parents	Homozygosity	Expected ratio	Observed ratio	Chi square	Sig/Non Sig at 0.01
	Blossom end shape	%	Flat: pointed(3:1)	Flat: Pointed	X^2	
RK1	F	100	7.5 : 2.5	10:00	3.33	NS
RK2	F	100	7.5 : 2.5	10:00	3.33	NS
RK3	F	100	7.5 : 2.5	10:00	3.33	NS
RK4	F	100	7.5 : 2.5	10:00	3.33	NS
RK5	S.P	60	7.5 : 2.5	6:04	1.2	NS
RK6	P	100	0:10	0:10	0	NS
RK7	F	100	7.5 : 2.5	10:00	3.33	NS
RK8	F	70	7.5 : 2.5	7:03	0.13	NS
RK9	F	50	7.5 : 2.5	5:05	3.33	NS
RK10	F	70	7.5 : 2.5	7:03	0.13	NS
RK11	F	70	7.5 : 2.5	7:03	0.13	NS
RK12	P	100	0:10	0:10	0	NS
RK13	S.P	60	7.5 : 2.5	6:04	1.2	NS
RK14	F	80	7.5 : 2.5	8:02	0.4	NS
RK15	F	100	7.5 : 2.5	10:00	3.33	NS
RK16	S.P	90	7.5 : 2.5	1:09	22.53	Sig
RK17	F	100	7.5 : 2.5	10:00	3.33	NS
RK18	F	100	7.5 : 2.5	10:00	3.33	NS
RK19	F	100	7.5 : 2.5	10:00	3.33	NS
RK20	P	80	7.5 : 2.5	8:02	0.4	NS
RK21	F	100	7.5 : 2.5	10:00	3.33	NS
RK22	S.P	70	7.5 : 2.5	3:07	10.8	Sig
RK23	P	100	0:10	0:10	0	NS
RK24	F	80	7.5 : 2.5	8:02	0.4	NS

F, flat blossom end; P, pointed blossom end; S.P semi pointed blossom end; NS, non-significant; Sig, significant The progeny of F_3 parental lines having flat blossom end RK1-4,7,15,17-19 and 21 showed non- significant difference between observed and expected values for blossom end shape in F_4 generation (Table 2). The lines did not segregate for the trait and produced only flat blossom end in F_4 generation. The lines have attained homozygosity for alleles for the trait in F_4 generation which is due to presence of flat blossom end alleles in homozygous condition.

The F_3 parental lines having flat blossom-end: RK8, 10 and 11 segregated into flat:pointed end in the ratio of 7:3, which is very close to expected values. The F3 parental lines (flat blossom end) RK 14,20 and 24 segregated into flat and pointed end in the ratio of 8:2. The ratio shows that flat blossom end genes were dominant; however, the lines were still heterozygous for the trait. Barten and Scott,[16] found that cross between pointed and flat blossom end tomatoes bear flat blossom end tomatoes in F_1 generation which shows that flat blossom end genes are dominant over genes responsible for pointed blossom end.

The F_3 parental line RK9 segregated into flat and pointed end blossom shapes in the ratio of 5:5, respectively. The intermediate expression and segregation may be due to the reason as reported by Rick,[17] that some genes for blossom end shape may also contribute intermediate expression for blossom end shape in heterozygous state.

Semi pointed blossom end parental lines RK5 and RK13 segregated into flat and pointed blossom end shapes in the ratio of 6:4 and showed no significant difference between observed and expected values. The result shows that there is dominance of flat blossom end alleles over pointed blossom end alleles. Other semi pointed-end F3 parental lines RK16 and RK22 segregated into flat and pointed end shapes in the ratio of 1:9 and 3:7, respectively .The F4 lines showed significant difference between observed and expected values. The ratios may be due to incomplete dominance for semi pointed end.

The F_3 parental lines with pointed end RK 6, 12 and 23 produced all the pointed blossom-end shaped fruits in F_4 generation and non-significant difference were observed between expected and observed values. The lines did not segregate further in F_4 generation because of presence of recessive genes for pointed end in homozygous condition. Barten et al.,[18] found that inheritance of pointed blossom end is due to recessive genes. However in heterozygous condition incomplete dominance can also be observed.

Conclusion

F_3 lines segregating in F_4 generation may deviate from the expected mendalian ratio for fruit shape. F_3 parents having obovoid-square shape may show homozygosity for most of the F_4 lines while F_3 parents having obovoid fruit shape may not show homozygosity in F_4 generation. Cylindrical F_3 parents can segregate into obovoid and pear shapes in F_4 generation. Flat blossom end shape is dominant over pointed end blossom shape.

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Conflicts of interest

The authors declared that there no conflicts of interest.

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